THE EFFECT OF RADIATED VS NON-IRRADIATED BLOOD TRANSFUSIONS ON EXTRACELLULAR POTASSIUM LEVELS IN INFANTS UNDERGOING CRANIOSYNOSTOSIS REPAIR

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Tyler Dunn
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Mentor: Raj Singhal, MD

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Abstract

Blood transfusions are associated with many types of risks including Transfusion Associated Graft Versus Host Disease (TA-GVHD), resulting when viable donor T-lymphocytes within the transfused product proliferate and attack host tissue. Efforts to address TA-GVHD are mostly focused on prevention as the disease has a high mortality rate (>90%) with limited options for effective treatment. An effective means to prevent TA-GVHD is the irradiation of cellular blood components which inactivate viable T-lymphocytes thereby preventing their proliferation. The downside to irradiating cellular blood products is that irradiation also affects other cellular components in the blood product causing the level of extracellular potassium to rise due to hemolysis of the red blood cells. In the pediatric population, rapid infusion of high potassium blood products has been associated with fatal cardiac arrhythmias. The objective of this study was to evaluate the effect of irradiated versus non-irradiated transfusions on extracellular potassium levels and to evaluate if washing irradiated blood prior to transfusion results in less of a change in extracellular potassium. A retrospective chart review was performed on 138 patients between two and ten months of age who had a craniosynostosis repair at Phoenix Children's Hospital from 2010 to 2015 and compared the extracellular potassium levels in patients under four months of age who received irradiated blood to patients over four months of age who received non-irradiated blood. A prospective blinded observational study in patients undergoing craniosynostosis repair was also performed in which ten patients less than four months of age received irradiated blood products and ten patients greater than four months of age received non-irradiated blood. The irradiated blood was washed within four to six hours of transfusion to remove potassium from the blood product. The patient's extracellular potassium measurement was recorded pre-transfusion, at thirty-minute intervals during the transfusion and post-transfusion. In the retrospective study there was a statistically significant ($p<0.001$) increase of 0.3 mmol/L in post-transfusion potassium levels of the irradiated ($n= 58$) group compared to the non-irradiated ($n= 80$). Pre-transfusion potassium levels were normally distributed; however post-transfusion values in the irradiated group were abnormally distributed. Additionally, 1.7% of the irradiated patients had a post-transfusion potassium level 5.5 mmol/L. In the prospective study there was statistically

significant ($p=0.004$) increase in the potassium level in the non-irradiated group ($n=10$) of 0.44 mmol/L at thirty minutes compared to the washed irradiated group of 0.19 ($n=9$) mmol/L. There is a greater difference in the pre-transfusion to thirty minute post-transfusion potassium level in the non-irradiated group of 0.55 mmol/L compared to 0.20 mmol/L in the washed irradiated group. There were no known adverse events in either study including the development of cardiac arrhythmia due to hyperkalemia. Irradiated blood is associated with a greater rise in extracellular potassium compared to non-irradiated but when an irradiated blood washing protocol is implemented prior to transfusion, there is not a significant rise in extracellular potassium. This is significant as TA-GVHD can be prevented with irradiation and the risk of hyperkalemia is minimized with washing prior to transfusion.

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Introduction/Significance

Blood transfusions may be necessary during surgery that involves a significant amount of blood loss. After homologous blood donation, whole blood is split into its components including platelets, cryoprecipitate, fresh frozen plasma, and red blood cells. Packed red blood cells (PRBC's), while principally composed of red blood cells, will still contain lymphocytes from the donor.[5] Lymphocytes are responsible for part of the body's immune system and are a component of the defense against foreign contaminants. By irradiating blood, there is a reduction in the rate of transfusion associated graft versus host disease (TA-GVHD) as the leukocytes responsible for the disease are destroyed.[2] This condition is rare with an incidence of 1 in 39,500 patients and has fallen significantly with the advent of more advanced blood bank practices and techniques.[3] TA-GVHD is more prevalent in patients from certain ethnicities (Japanese) and patients that are immunocompromised.[3] While there are no definitive studies outlining when children have a fully functional immune system, many report that children under six months of age are a more vulnerable population for TA-GVHD.[1]

Irradiation of blood, to reduce the risk of TA-GVHD, also causes the level of extracellular potassium to rise due to hemolysis of the red blood cells.[6] When this blood is transfused, it can result in increased extracellular concentrations of potassium. When extracellular potassium rises too quickly or becomes higher than 6.5 meq/L, patients are at significant risk for hemodynamic changes, cardiac arrhythmias, and potentially cardiac arrest.[4,7] Increased extracellular potassium levels interfere with the conduction system of the heart due to imbalanced cellular signals which results in these arrhythmias. Hyperkalemia from rapid transfusion occurs much more frequently than TA-GVHD, but like TA-GVHD, there is a tendency to under-report this complication.[8]

In order to reduce the incidence of this complication, blood can be transfused slowly, at a rate of less than 5 ml/kg/min. In emergency situations or in states where there is rapid blood loss, the infusion rate may exceed the safe limit which then overwhelms intracellular mechanisms to absorb the additional potassium load. In this case, administration of calcium can help with preventing cardiac conduction anomalies.[8]

After a comprehensive review of the relevant literature, there is a lack of information comparing the effects of non-irradiated and irradiated blood on the extracellular potassium levels of the patient. There are studies that measure the amount of potassium in PRBC's and show that there is a statistically significant increase in potassium of irradiated units versus non-irradiated units.[9] Other studies show that excessively fast transfusion rates (greater than 1 ml/kg/min) can result in increased extracellular potassium levels and cardiac conduction issues. There are no studies, however, that differentiate differences in extracellular potassium during the transfusion of irradiated versus non-irradiated PRBC's.

In this particularly vulnerable population of children under six months of age, there have been instances of cardiac arrest during transfusion secondary to hyperkalemia as the practice is to transfuse irradiated units of blood in an effort to prevent the occurrence of TA-GVHD. Specifically, in patients undergoing a craniosynostosis repair, the rate of transfusion can be high, with blood loss that can range between one-half to two blood volumes.[10] With rapid transfusion of irradiated blood, these patients receive a significant load of potassium, which may be more clinically significant than the risk of TA-GVHD. We propose a study, on patients undergoing craniosynostosis repair, to observe the change in extracellular potassium during transfusion, immediately after completion of transfusion, and thirty minutes after the end of the transfusion in patients who receive irradiated blood compared to those who receive non-irradiated blood.

Materials and Methods

This study included both a retrospective chart review of 138 patients as well as a prospective blinded observational study with 20 patients who all underwent craniosynostosis repair. The Phoenix Children's Institutional Review Board approved both studies. Patients received blood products intraoperatively per the current standard of care at Phoenix Children's Hospital at the discretion of the anesthesiologist and surgeon. Namely, children under four months of age received irradiated blood products and children over four months of age received non-irradiated blood products. Patients who declined study enrollment had no change in their anesthetic and post-operative management and no form of therapy was intentionally withheld.

Study items collected and documented for all patients enrolled in both studies included patient age, sex, weight, type of blood transfused (ABO and irradiated/non-irradiated), rate of transfusion, vital signs, operating time, amount of blood lost, amount of calcium administered, extracellular potassium levels (at intervals indicated), adverse events, and length of stay.

Retrospective Chart Review:

This retrospective study included 138 patients between two and ten months of age who had a craniosynostosis repair and required a blood transfusion at Phoenix Children's Hospital between January 2010 and August 2015. Data was collected from anesthesia records, blood bank documentation, and the EMR including patient's extracellular potassium measurement recorded pre-transfusion, during transfusion and post-transfusion.

Prospective Blinded Observational Study with Washed Irradiated Blood:

Patient enrollment in the study occurred until twenty patients were enrolled, ten patients per arm. Families who agreed to enroll patients under ten months of age were assigned to either category:

1) <4 months of age received irradiated blood products which were washed with normal saline prior to transfusion

2) >4 months of age received non-irradiated blood products

The study was blinded to both the surgeon and the anesthesiologist and all blood collected for analysis was done via arterial lines. All blood (PRBC's) transfused was analyzed prior to transfusion and the patient's extracellular potassium measurement was recorded at thirty-minute intervals during the transfusion as well as pre-transfusion and post-transfusion.

Statistical Analyses:

The distribution of demographic and baseline characteristics were summarized using counts and percentages for categorical variables, and the mean and standard deviation or the median and interquartile range for continuous measurements. Differences in the distribution of baseline variables were compared between patients who received irradiated or non-irradiated blood transfusion using the Pearson Chi-square test for categorical variables and ANOVA for continuous variables. Changes in the extracellular potassium levels were computed as the difference between measurements obtained post and pre-surgery. The change in potassium was compared between irradiated and non-irradiated blood groups using the T-test or Kruskall-Wallis Test overall and within weight categories. In addition, multivariable linear regressions estimate the association of irradiated or non-irradiated blood product with change in potassium level, after adjustment for confounders including age and weight as well as other important risk factors. All statistical tests are 2-sided with significance evaluated at the 5% level. The data was compared to determine if there is any correlation between extracellular potassium levels, the type of blood transfused, and the amount of calcium administered.

Results

Retrospective Chart Review:

There were 80 patients that received non-irradiated blood and 58 patients that received irradiated blood with a statistically significant difference of 0.3 mmol/L ($P<0.001$) in mean post-transfusion potassium levels (non-irradiated= 3.85 mmol/L, irradiated= 4.15 mmol/L). Moreover, there is also a statistically significant difference in the medians (non-irradiated= 3.80 mmol/L, irradiated= 4.05 mmol/L). When looking at mean potassium levels during blood transfusion based on the first arterial blood gas drawn during transfusion there is a marked increase of 0.31 mmol/L in the irradiated patients at 3.99 mmol/L compared to non-irradiated at 3.68 mmol/L which is statistically significant ($P<0.001$).

Normal probability plots and tests for normality (Anderson-Darling Test statistics) were conducted to test for normal or non-normal distributions. All pre-administration potassium levels were normally distributed. Post-transfusion potassium levels for non-irradiated patients were also normally distributed while irradiated patient levels were not, having a few extreme values with 1.7% of irradiated patient having a post-transfusion potassium level $\geq$ 5.5 mmol/L.

Subtracting the post-transfusion potassium levels from the pre-transfusion potassium levels for the both the irradiated and non-irradiated groups allowed an examination of the mean and median differences for these groups which shows that the overall mean differences in pre vs. post-transfusion potassium levels are almost identical.

When looking for a correlation in change of potassium levels pre and post transfusion with cc's/kg/min there was greater variability in the irradiated levels while the non-irradiated group was more tightly clustered. There was no significant linear correlation between the two variables with a Pearson correlation of 0.163 and when three outliers are removed the Pearson correlation was still poor at 0.144.

	Non-Irradiated	Irradiated
Mean Post Transfusion	3.85	4.15
Median Post Transfusion	3.80	4.05
Mean First ABG	3.68	3.99
Standard Deviation	0.45	0.51
Distribution	Normal	Non-Normal

Table 1: Retrospective Potassium Levels

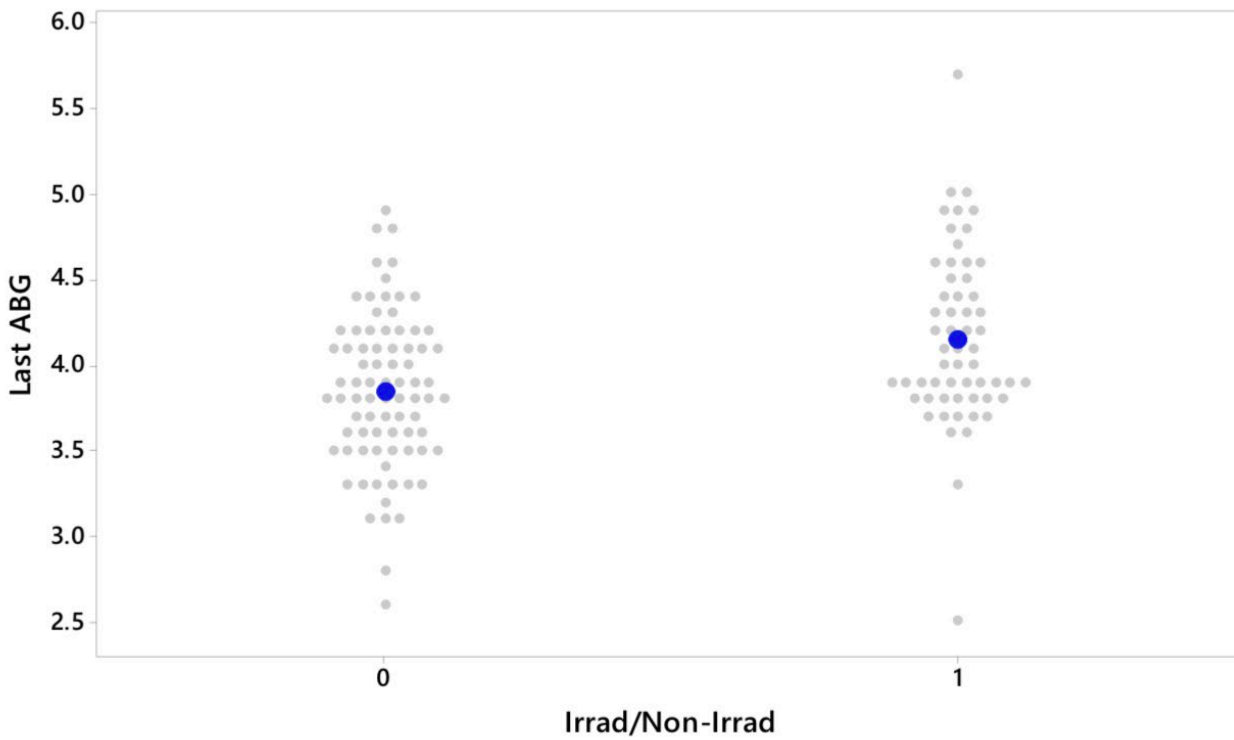


Figure 1: Plot of Last Potassium Level after Transfusion, (0=Non-Irradiated, 1=Irradiated)

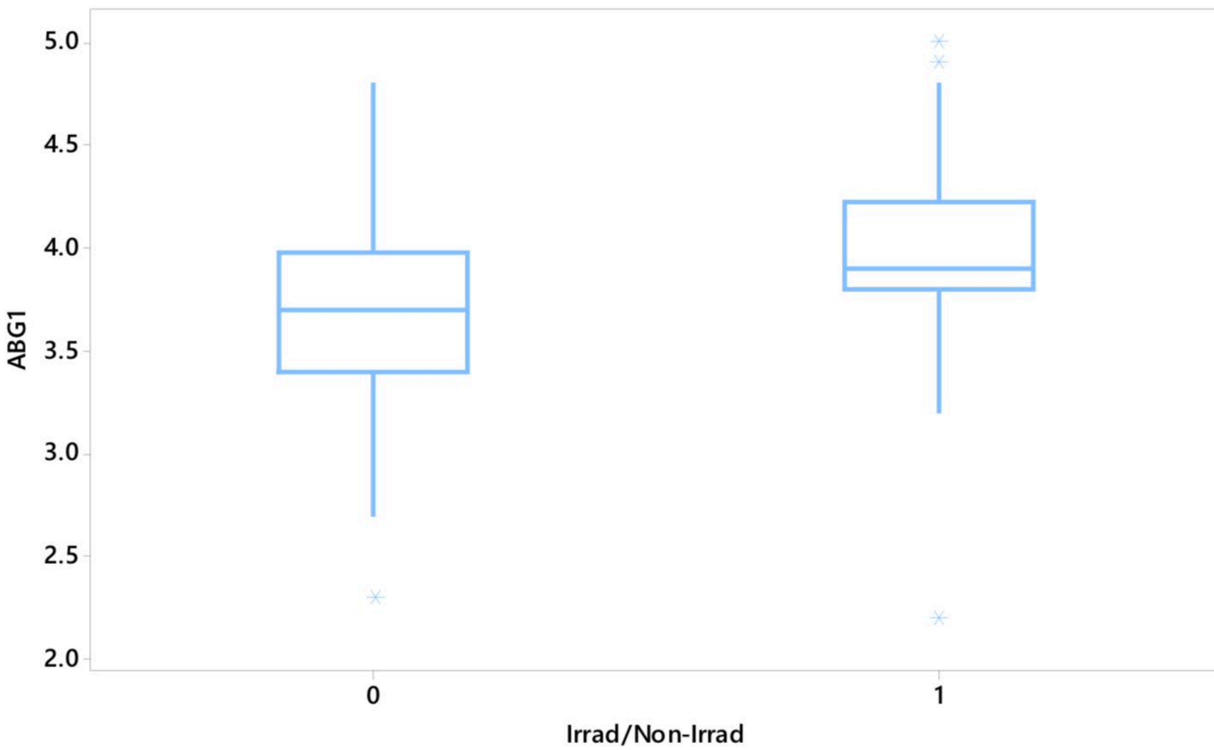
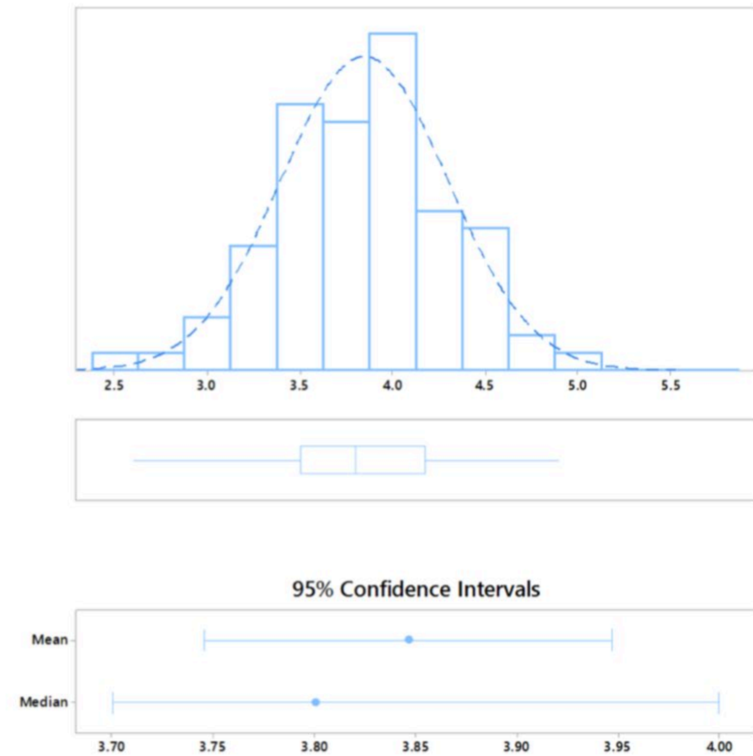
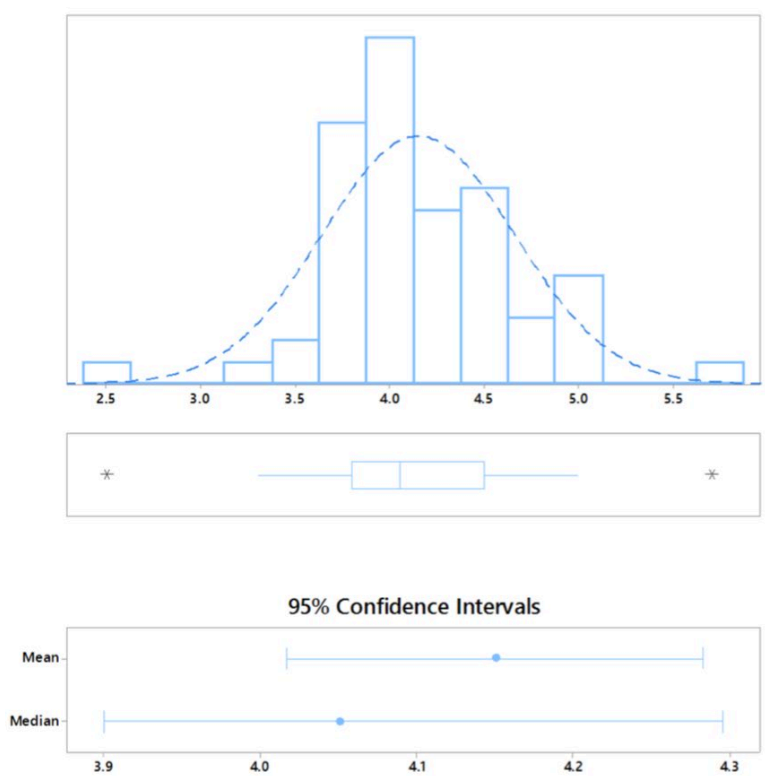


Figure 2: Boxplot of First Potassium Level during Transfusion, (0=Non-Irradiated, 1=Irradiated)



Anderson-Darling Normality Test	
A-Squared	0.29
P-Value	0.605
Mean	3.8462
StDev	0.4517
Variance	0.2040
Skewness	-0.080210
Kurtosis	0.112192
N	80
Minimum	2.6000
1st Quartile	3.5000
Median	3.8000
3rd Quartile	4.1750
Maximum	4.9000
95% Confidence Interval for Mean	
3.7457	3.9468
95% Confidence Interval for Median	
3.7000	4.0000
95% Confidence Interval for StDev	
0.3909	0.5350



Anderson-Darling Normality Test	
A-Squared	1.08
P-Value	0.007
Mean	4.1500
StDev	0.5069
Variance	0.2569
Skewness	0.15110
Kurtosis	1.82235
N	58
Minimum	2.5000
1st Quartile	3.8000
Median	4.0500
3rd Quartile	4.5000
Maximum	5.7000
95% Confidence Interval for Mean	
4.0167	4.2833
95% Confidence Interval for Median	
3.9000	4.2954
95% Confidence Interval for StDev	
0.4285	0.6206

Figure 3: Anderson-Darling Normality Test of Last Potassium Level after Transfusion in Patients Receiving Non-Irradiated (top) and Irradiated (bottom) Blood

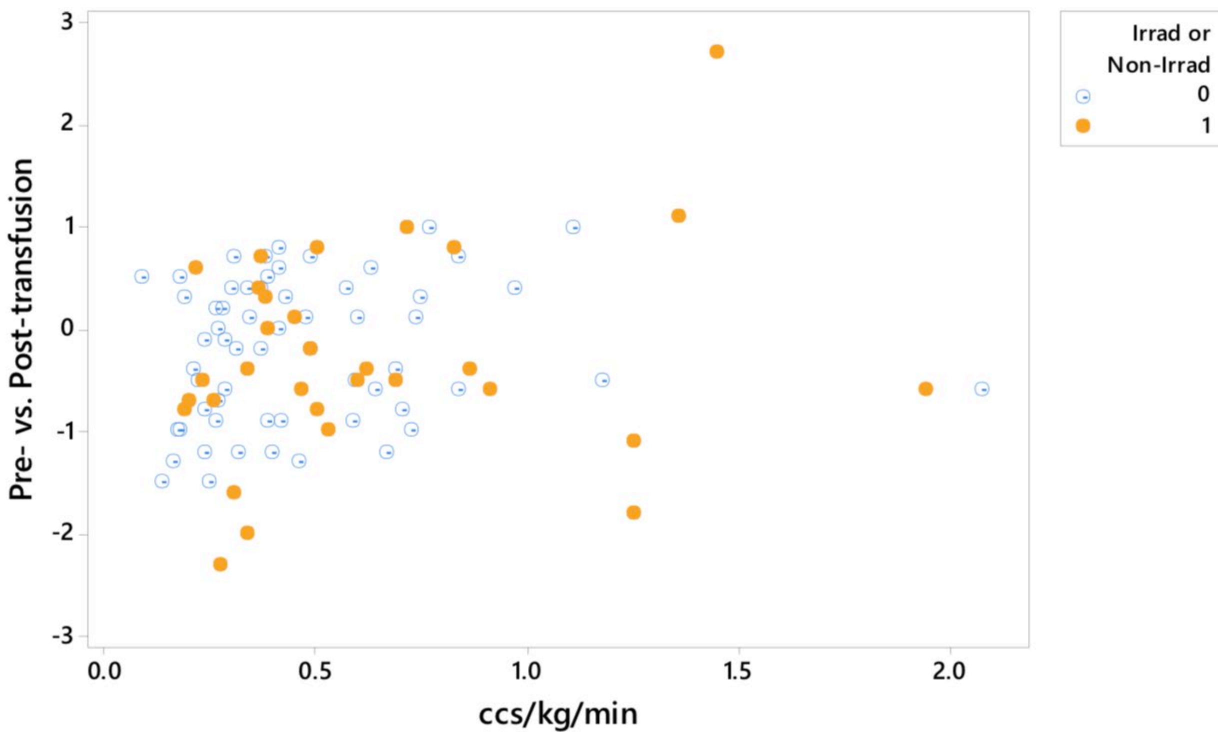


Figure 4: Scatterplot of Pre vs Post Transfusion Potassium Level vs cc's/kg/min, (0=Non-Irradiated, 1=Irradiated)

Prospective Blinded Observational Study with Washing of Irradiated Blood:

In both groups the mean potassium levels at 30 minutes post transfusion were similar at 3.93 mmol/L for non-irradiated and 3.99 mmol/L for the irradiated group. There is a greater median difference in the pre-transfusion to 30 minute post-transfusion potassium level in the non-irradiated group of 0.55 mmol/L compared to 0.20 mmol/L in the washed irradiated group.

When evaluating for any correlation of change in potassium levels pre vs 30 minute post transfusion with total amount of blood transfused (cc's/kg) there was no linear correlation observed with a Pearson correlation of -0.040 (p=0.870). However, there was a weak positive linear relationship with rate of transfusion (cc/kg/min), Pearson correlation of 0.213, but not found to be statistically significant (p=0.381). When comparing pre-transfusion potassium levels of irradiated versus non-irradiated patients using a two-tail (p-value = 0.100) and Mann-Whitney test (p-value = 0.070), there was no significant differences between the two groups and the initial potassium levels were representative of mere sampling variation. There were no known adverse events in either study including the development of cardiac arrhythmia due to hyperkalemia.

Mixed-effects models were conducted to determine if the study design itself, (irradiated vs. non-irradiated groupings, additional bags given, or if random effects of patient physiologies) would generate significant differences in the k-levels seen in this pilot study. After four mixed models were conducted, the only significant factor contributing to differences in k-levels were the various and random individual patient physiologies themselves. Neither the study groupings nor the additional bags given to five of the patients revealed any significant contributions to differences in k-levels.

	Non-Irradiated	**Irradiated**
Mean Potassium 30 min Post Transfusion	3.93	3.99
Mean Difference in Potassium Levels Pre to 30 Minute Post Transfusion	0.55	0.20

Table 2: Prospective Potassium Levels with Washing

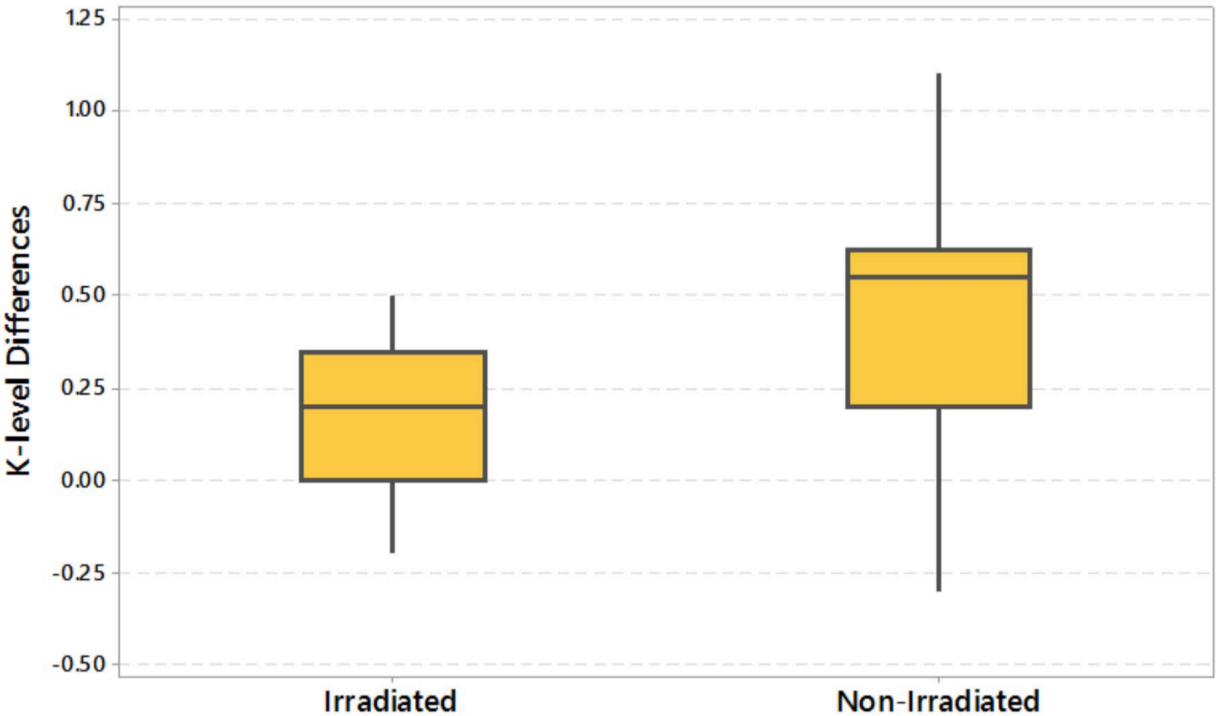


Figure 5: Differences in Potassium Levels at 30 Minutes Post Transfusion Compared to Pre-Transfusion

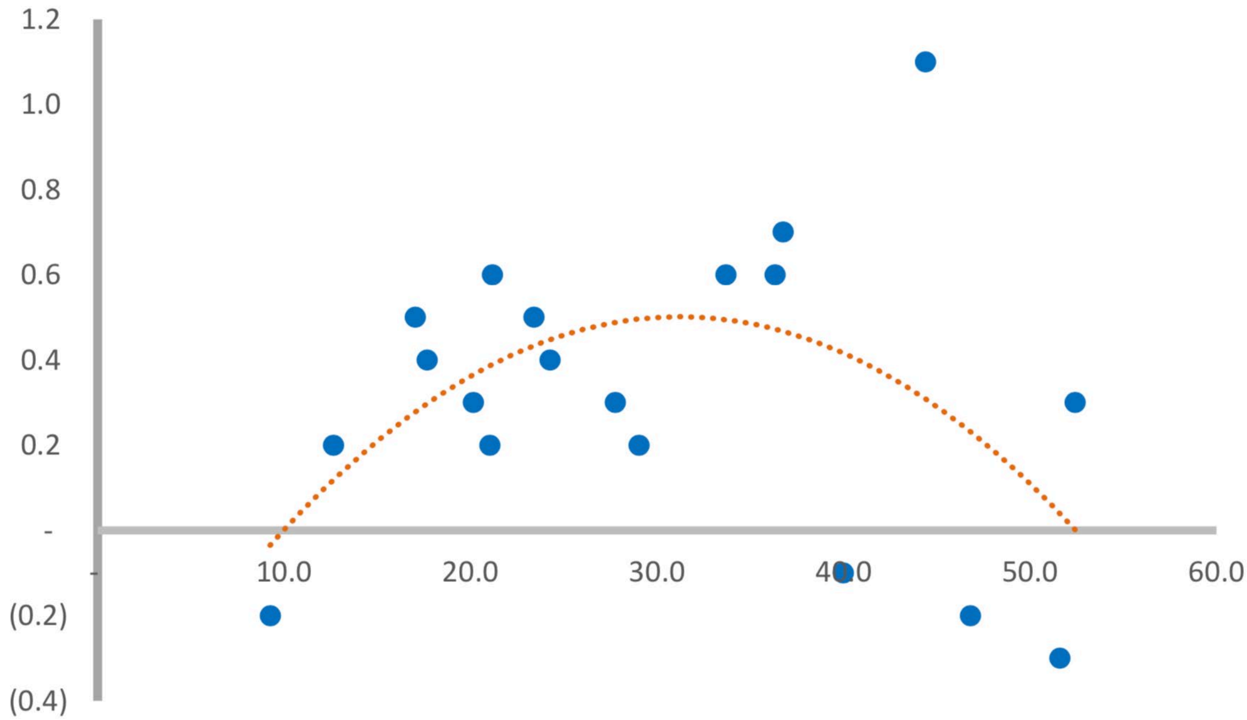


Figure 6: Volume of Transfusion, cc/kg, (X Axis) vs Potassium Level Difference 30 Minute Post Transfusion (Y Axis)

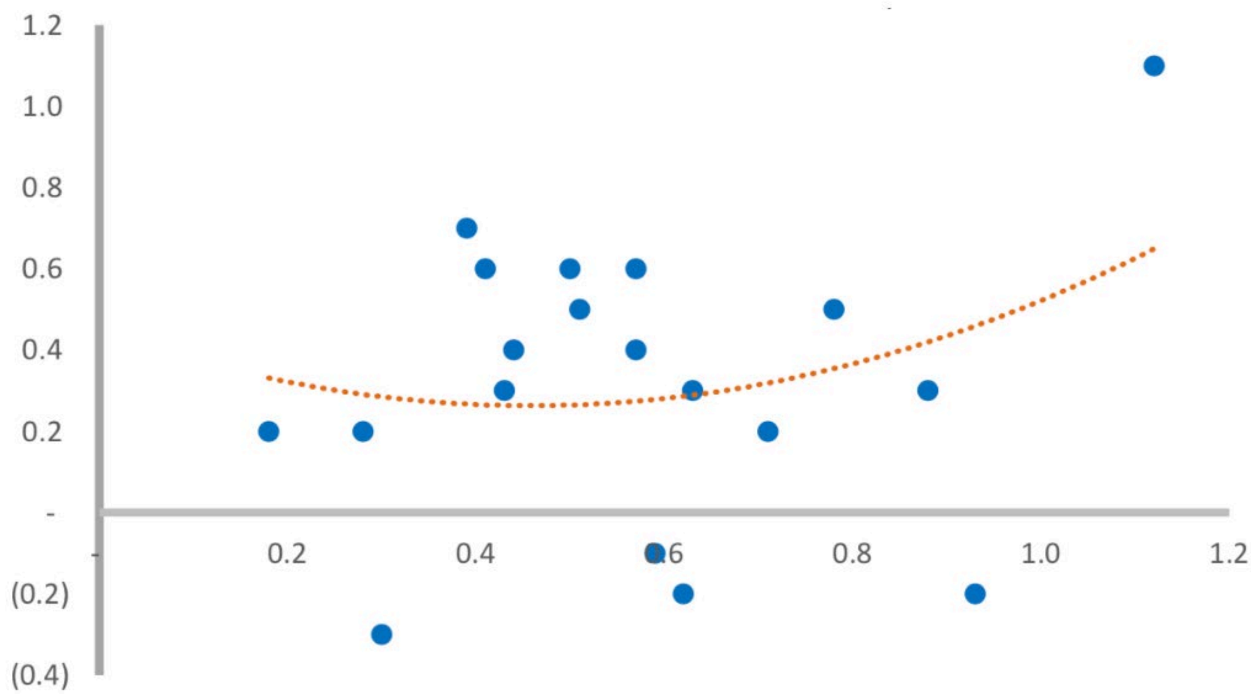


Figure 7: Rate of Transfusion, cc/kg/min, (X Axis) vs Potassium Level Difference 30 Minute Post Transfusion (Y Axis)

Discussion

The retrospective study is the first study to quantify the rise in extracellular potassium immediately after transfusion with respect to irradiated and non-irradiated blood, in which there is a clear rise in extracellular potassium with irradiation of blood. The distribution of potassium levels post transfusion in the irradiated group is non-normal with a skew to the right suggesting that when blood is irradiated there is some uncertainty in the degree of hyperkalemia which could pose a threat to a patient receiving large quantities of irradiated blood. While 1.7% of irradiated patients had a post-transfusion potassium level $\geq$ 5.5 mmol/L which could be significant for EKG changes in the infant population this was not observed. When looking at the correlation in change of potassium levels pre and post transfusion with cc's/kg/min there was not a strong relationship although there was more variability with irradiated blood suggesting that the development of hyperkalemia may be more related to volume of blood received and not the speed of transfusion. The data for cc's/kg/min may also be skewed as the time that a transfusion was given over was rounded to the nearest 15 minutes due to limitations in paper anesthetic record and this allows for little variability in transfusion duration.

In the prospective study with the washing of irradiated blood before transfusion, 30-minute post transfusion potassium levels were very similar between the irradiated and non-irradiated groups. This suggests that the washing of irradiated blood with normal saline prior to transfusion is a successful way to minimize the negative effect of hyperkalemia while still irradiating the blood to prevent TA-GVHD. While washing irradiated blood does take extra time, manpower, and has an increased cost this study does show that there is benefit in doing this. In the study there was a greater median difference in the pre-transfusion to 30-minute post-transfusion potassium level in the non-irradiated group suggesting that in non-irradiated blood transfusions there is a still a rise in potassium from lysis of cells in the blood product. In the prospective study there was lack of evidence that the volume of blood transfused had any correlation in potassium levels but there is a weak correlation with rate of transfusion, but this needs to be further investigated as this was not the main aim in our study and would need alteration of the protocol to capture the best data for this.

Future Directions

For future studies I would like to measure potassium levels in each unit of blood prior to transfusion to assure that they are similar prior to transfusing when comparing irradiated non-washed blood and non-irradiated. This will assure significant differences do not change the post transfusion potassium levels. Another future study would be to repeat the retrospective study now that an electronic anesthetic record has been implemented at PCH to see if there is a correlation with potassium levels and speed of transfusion as now this software captures transfusion duration to the nearest minute compared to 15 minute intervals with the previous paper charting.

Conclusions

Irradiated blood is associated with a greater rise in extracellular potassium compared to non-irradiated but when an irradiated blood washing protocol is implemented prior to transfusion, there is not a significant rise in extracellular potassium. This is significant as TA-GVHD can be prevented with irradiation and the risk of hyperkalemia is minimized with washing prior to transfusion.

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